Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Mgr. Ivana Brabcová

Supervisor: Doc. RNDr. Dalibor Šatínský, Ph.D.

Title of thesis: The use of modern chromatographic approaches and sample preparation in the analysis of biologically active substances

The presented thesis deals with the modern trends in liquid chromatography and in the analysis of biologically active substances. High performance liquid chromatography (HPLC) is an important analytical tool for separating and quantifying components of mixtures.
New trends of HPLC technology tend to development of new stationary phases with better properties. Another trend in analytical chemistry is a miniaturization of the HPLC system and environmentally friendly chromatography.

Sample preparation is an essential step in analysis, greatly influencing the reliability and accuracy of analysis. Nowadays, the development is focused on new modern techniques of sample preparation. The developments of these techniques lead up to miniaturization, automatization and preconcentration of target analytes.

The theoretical part of the thesis deals with the introduction of modern trends in liquid chromatography and it is divided in several sections.

First part describes current trends in fast liquid chromatographic separations and monolith technology, fused core columns and sub-2-micron particles columns. A further development is focused on stationary phases which are suitable chromatographic properties, i.e. the ability to better separation of the over a wide pH range and at elevated temperatures.
Another chapter of the theoretical part is devoted to techniques of sample preparation for analysis. Sample preparation is an important step, the implementation of which depends on the accuracy of the result. Commonly used traditional techniques of sample preparation are described. Attention is paid to modern extraction techniques, which are developing in recent years.

The last part of the theoretical work deals with food supplements. Currently, there is a developing market for food supplements that contain concentrated sources of biologically active substances. The products containing biologically active substances are commercially available in two forms, as registered medicaments and as food supplements. This chapter is

focused on legislative differences between medicaments and food supplements and the resulting differences in quality control is the focus of this chapter. The experimental part of the work dealing with two of the work of sample preparation and the development of methods for the determination of vitamins in food supplements.

The experimental part of the thesis is divided into three thematic areas.

The first topic deals with column switching technique. It is presented in two works that have been developed and validated. In the first case, is work the determination of beta-carotene in food supplements. The next work deals with the determination of non-steroidal anti-inflammatory drugs in human serum.

The second area focuses on the use of different stationary phases in chromatographic analyses.Thie section contains three works. The first work deals with the development and optimization of methods for the determination of vitamin E acetate in various food supplements. Next work deals with the development and optimization of methods for determining fenbendazole, praziquantel, pyrantel pamoat and oxfendazole, which are used to eliminate parasitic worms in human and veterinary medicine.

Another work deals with the development of methods for the determination of ascorbic acid, phenylephrine, paracetamol and caffeine. In this work, were compared, monolithic columns with different dimensions.

The last chapter deals with the issue of "green" chromatography. The aim of the first work was to design an optimal chromatographic conditions for the determination of noradrenaline. And the second paper deals with the development and validation of HPLC method for determination of 4-aminophenol, caffeine, paracetamol and propyphenazone.

The developed methods are described in published works. Their full texts are listed in the Appendix and are accompanied by a brief commentary in the "Results and discussion".